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RESEARCH ARTICLE

Development of the interscutularis model as an outcome measure for facial nerve surgery

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ABSTRACT

Introduction: Animal models for the study of facial paralysis have been well developed, but concern has arisen regarding the accuracy of eye closure and whisker movement as outcome measures due to new data regarding interconnectivity between facial nerve branches and autonomic innervation. The posterior auricular nerve (PAN) is an isolated branch of the facial nerve which has been confirmed as the sole motor innervat or of the interscutularis muscle. This study was designed to develop a model for facial nerve palsy utilizing the PAN and interscutularis muscle.
Methods: A custom-made automated video capture system was built into a poly methyl methacrylate cage using a high definition monochrome digital camera and image sensor to record the animal as it drank from a water feeder. A copper floor pad and copper collar around the water feeder were connected to an electrical circuit for automatic saving of the video recording 10 s prior to and 30 s following the drinking event. A pre-operative baseline recording of ear movement during drinking was captured. Female YFP-16 mice at 6 weeks were assigned to sham (Sh, n = 5), nerve excision (Ex, n = 10), or nerve crush (Cr, n = 10) groups with all interventions performed on the right PAN. Sh mice were irrigated with 10 ml normal saline as were the Ex and Cr mice following operative intervention. In Ex mice, a 3 mm section of the PAN was sharply excised and nerve gap was confirmed with fluorescent microscopy. In Cr mice, the PAN was crushed 3 mm from the origin of the facial nerve trunk with size 5 jeweler's forceps for two periods of 20 s. Post-operative video recordings were collected on post-operative days (POD) 1, 10, 20, and 30. To determine the change in ear movement, the right ear was graphically compared to the left control side.
Results: Sh animals exhibited a statistically significant reduction in ear movement at POD01 compared to other POD recordings ($p < 0.05$), but no significant change in right ear movement following POD05. Ex animals had a significant reduction in right ear movement at all PODs in comparison to the left ear ($p < 0.05$) with no significant change in right ear movement during the study period ($p = 0.94$). Cr animals showed a significant reduction in right ear movement compared to the left at POD01, POD10, and POD20 ($p < 0.05$). At POD30, there was no significant difference between ear movement on either side ($p = 0.35$). There was a significant change in right ear movement during the data collection period ($p < 0.05$).
Conclusion: The results show that significant differences were demonstrated between the experimental groups and that significant changes within the crush group were identifiable making this an acceptable model to develop as an accurate outcome measure following rodent facial nerve surgery.

1. Introduction

Outcome measures are crucial to reporting results of interventions studied in facial paralysis. Since the original monitoring system proposed by House (1983), more than 25 outcome measures have been developed for the evaluation of facial paralysis (Fattah

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et al., 2015; Guarin et al., 2018; Niziol et al., 2015) including fully automated measurement systems (Dorschner et al., 2016); however, the interconnections between facial nerve branches and lack of emotive testing has created a need for a more accurate animal model.

1.1. Animal models for assessment of facial nerve surgery outcomes

Animal models for the assessment of nerve regeneration are well established, with most models centered on peripheral nerve injury to the hind limb (Wood et al., 2011). Whilst these systems have been well studied and offer good inter-user reliability, there are fundamental differences between the regenerative properties of central nervous system (CNS) axons and peripheral nervous system (PNS) axons (Huebner and Strittmatter, 2009). Although the facial nerve exits the cranium and has many similarities to a peripheral nerve, its cell bodies remain in the brainstem located in the facial motor nucleus. Studies demonstrate that the CNS environment is inhibitory to axon growth while the PNS environment is stimulatory (Aguayo et al., 1991; Benfey and Aguayo, 1982; Bray et al., 1987; David and Aguayo, 1981); therefore, the supposition that the facial nerve behaves exactly as a peripheral nerve may be inaccurate. Animal models specific to facial nerve assessment exist for this reason.

In rodents, studies have utilized two main outcomes as markers for facial nerve function and regeneration: eye closure and whisker/vibrissae movement (Angelov et al., 2007; Bischoff et al., 2009; Grosheva et al., 2008; Guntinas-Lichius et al., 2001, 2005; Guntinas-Lichius et al., 2002; Hadlock et al., 2008, 2010; Heaton et al., 2014; Hohman et al., 2014; Pavlov et al., 2008; Tomov et al., 2002). While initial studies promoted the implementation of whisking or eye-closure assessment (Angelov et al., 2007; Bischoff et al., 2009; Grosheva et al., 2008; Guntinas-Lichius et al., 2001, 2005; Guntinas-Lichius et al., 2002; Hadlock et al., 2008, 2010; Hohman et al., 2014; Olmstead et al., 2015; Pavlov et al., 2008; Tomov et al., 2002), these have largely been retracted (Heaton et al., 2010, 2014; Henstrom et al., 2012b), citing alternative mechanisms allowing movement to occur independent of the facial nerve. Henstrom et al. (2012b) additionally demonstrated extensive cross-innervation between most facial nerve branches in Wister rats, and further work showed that observable vibrissal movement occurs with stimulation of marginal mandibular, buccal, distal pes, or individual distal pes branches, indicating that these motions are not branch-specific (Henstrom et al., 2012a).

1.2. The posterior auricular nerve and interscutularis muscle as a new model for facial nerve study

One potential solution to the aforementioned interconnectivity challenge is the use of a nerve branch with isolated function and without cross-innervation. The posterior auricular nerve (PAN), believed to innervate the interscutularis muscle, was evaluated (Lu et al., 2009). In this study, the PAN was mapped in a three-dimensional structure known as a connectome for full visualization. This connectome showed all motor innervation to the interscutularis muscle originated from the PAN; the PAN was traced from the muscle and was not found to have any collateral connections to other branches of the facial nerve. As such, the function of the interscutularis muscle may be used as an indicator of PAN function and, by extension, facial nerve function. The interscutularis muscle, while not previously well studied, is anatomically associated with the ear. Animal behavioral research has demonstrated that ear position can convey the animal's situational behavior. Behavioral studies in sheep have reported ear-position as a reliable indicator of suffering (Stracke et al., 2011; Stubsjoen et al., 2009). If animals were distressed or in pain, the ear posture was more likely to be retracted and furthermore, was shown to convey positive and negative emotion (Martin and Bateson, 2007; Reefmann et al., 2009; Schmied et al., 2008). Anticipated emotional reaction to known stimuli may allow for a predictable route of evaluation for PAN and interscutularis muscle function.

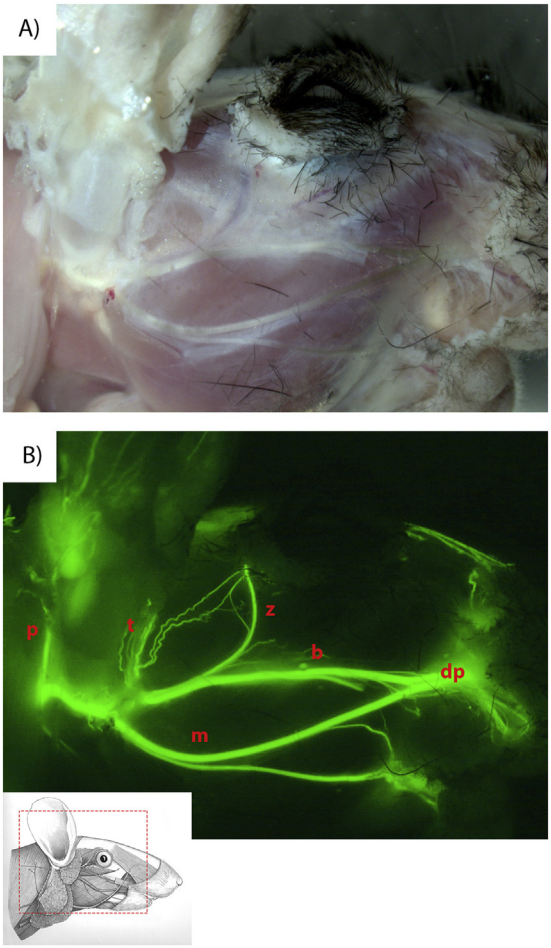

Fig. 1. Anatomical dissectionof the facial nerve of a mouse. (A) The superficial anatomy of the face of a YFP-16 mouse. The skin has been removed to expose the underlying facial nerve and musculature. The main branches of the facial nerve can be appreciated. (B) The same animal under fluorescent imaging. Under these conditions the cross-innervation patterns may be fully appreciated. Cross-innervation can be seen between the temporal and zygomatic branches, between the zygomatic and buccal branches and extensively between the buccal and mandibular branches; no demonstrable cross-innervation was seen with the posterior auricular nerve (PAN). [*Key*: p — posterior auricular nerve, t — temporal branch, z — zygomatic branch, b — buccal branch, m — mandibular branch, dp — distal pes].

1.3. Preliminary study: extra-cranial anatomy of the facial nerve in the laboratory mouse

Firstly, in order to verify that the PAN has no cross-connectivity with other facial nerve branches, dissection of the facial nerve in five three-month old YFP-16 transgenic mice was performed under the guidance of a fluorescent macro-zoom microscope. The facial nerve was seen to emerge from the cranium via the stylomastoid foramen, approximately 12 mm inferior and 5 mm posterior to the most inferior aspect of the auricle. The branching pattern was consistent in all animals. The first branch, the posterior auricular branch, arose as the facial nerve emerged from the stylomastoid foramen, and coursed immediately behind the auricles to innervate the interscutularis muscle. The facial nerve trunk proceeded

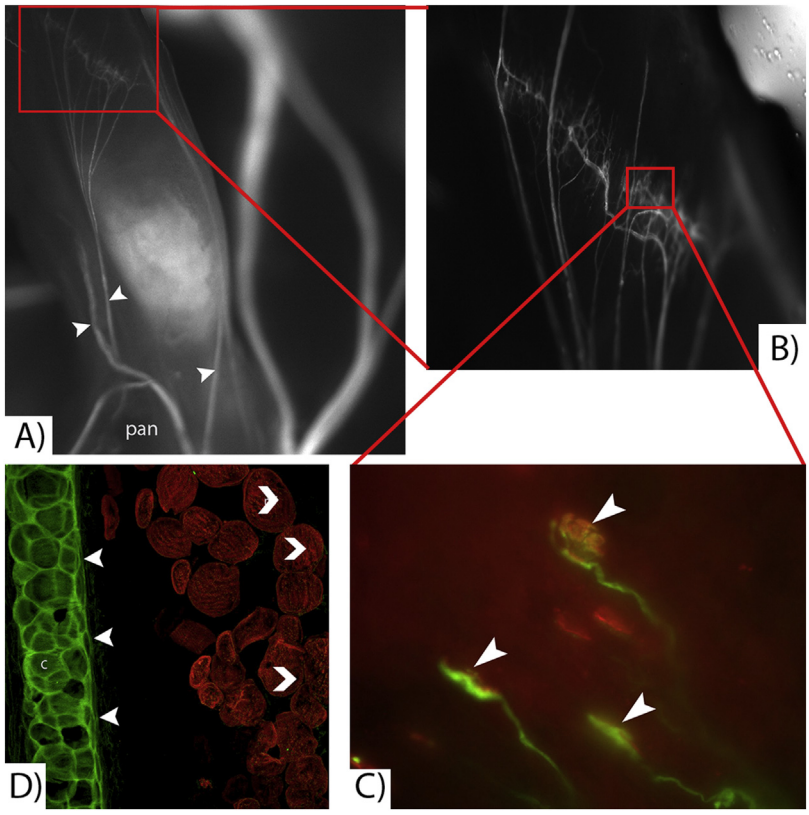

Fig. 2. Anatomical and immuno-histological study of the mouse ear. (A and B) The posterior surface of the mouse ear following de-epithelialization. Three discreet branches of the posterior auricular nerve PAN) [white arrowheads] were consistently observed to form a band of neuromuscular junctions (NMJs). (C) α-Bungarotoxin was used to confirm the presence of NMJs [white arrowheads]. (D) Cross-section of mouse ear tissue. Staining with anti-MyHC2B (Life Technologies, Carlsbad, CA, USA) demonstrated the presence of type IIB muscle fibers (white chevron markers) associated with auricular cartilage (white arrowheads). These finding support the discovery of a previously unreported muscle of the mouse ear.

towards the face and separated into three divisions: the temporal, the zygomatic/buccal and the mandibular/cervical divisions. Continuing distally, these divisions separated into their terminal branches — the temporal, zygomatic, buccal, mandibular and cervical. However, several interconnections were noted between these terminal branches. With the assistance of the fluorescence microscope and YFP-16 transgenic animals, cross-innervation in all areas was clearly demonstrated (Fig. 1).

The posterior auricular branch had no identifiable cross-innervation with any other facial nerve branch. The nerve passed deep to the greater auricular nerve and was noted to give off three consistent branches to the posterior surface of the auricle, thus forming discrete neuromuscular junctions (NMJs) in a banded fashion at the distal third of the auricle. Immunohistological α-bungarotoxin staining confirmed these findings (Fig. 2). The PAN continued distally as a terminal branch to supply the interscutularis muscle.

1.4. Preliminary study: function of the posterior auricular nerve

Secondly, to evaluate the function of the PAN, the posterior auricular nerve was surgically isolated in six YFP-16 mice. Nerve stimulation (Vari-Stim® III, Medtronic, Xomed Inc.) at the origin of the PAN resulted in retraction of the auricles posteriorly with flattening towards the skull. When stimulated distal to the three auricular branches, only retraction of the ear could be demonstrated, suggesting the three previously undefined branches supplying the posterior auricular region are responsible for the curling of the auricle. Thus, it was concluded that PAN functions to cause auricular retraction posteriorly via contraction of the interscutularis muscle.

To understand the consequence of injuring the PAN, each animal had the right nerve segmentally excised. The skin incision was closed with absorbable sutures, and the animals were allowed to recover from surgery. Assessment of the animals post-operatively showed the right ear was held in a forward/ventral posture and was not retracting posteriorly in comparison to the contra-lateral side (Fig. 3E and F).

1.5. Development of a new model for evaluation of facial nerve injury and surgical repair

With confirmation that the PAN harbors no cross-innervation with other facial nerve branches and that it has a clear, identifiable function, the investigators set forth to develop an ideal animal model for facial nerve injury and surgical repair utilizing the PAN and interscutularis muscle. The objectives were as follows:

Aim 1: establish a method to reliably measure ear movement and, therefore, indicate the level of PAN functionality.

Aim 2: perform controlled, reproducible experiments in order to establish PAN injury as an accurate and measurable indicator of facial nerve injury with previously defined outcome measures.

2. Methods

2.1. Automated video capture of ear movement

To record changes in ear movement, a custom video-capture suite was fabricated based around the methodology described by Heaton et al. (Heaton et al., 2008). A colorless round poly methyl methacrylate cage was created measuring 30 cm in diameter with

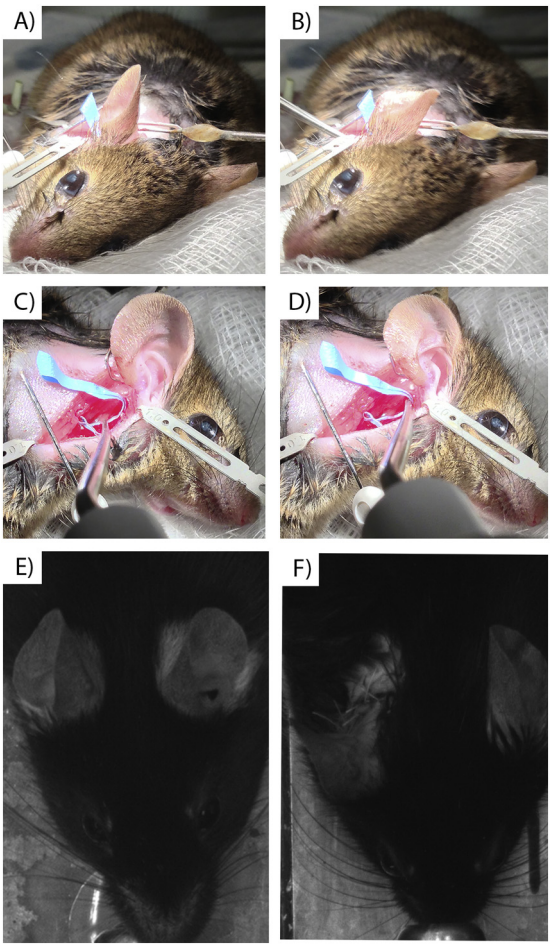

Fig. 3. Function of the posterior auricular nerve in a mouse. (A) The anterior position of the right ear prior to stimulation of the posterior auricular nerve. (B) During stimulation of the posterior auricular nerve, the ear can be seen to retract towards the mid-line. (C) The lateral position of the ear at rest. (D) During stimulation of the posterior auricular nerve, the ear is retracted posteriorly. (E) Baseline video still of a mouse recorded whilst they drank from the water feeder in the fabricated video-suite. (F) Post-operative video still following excision of the right posterior auricular nerve demonstrating the inability to retract the ear.

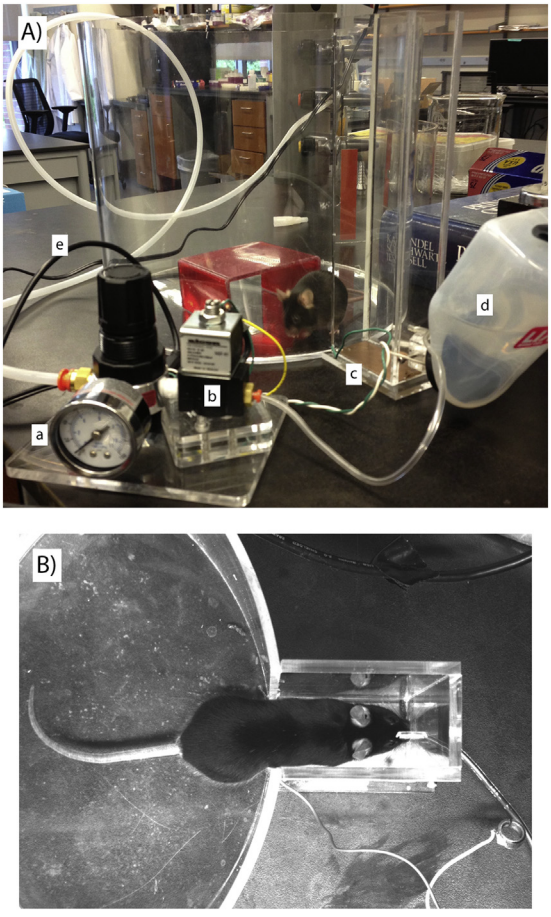

Fig. 4. Overview of the video-suite used to assess ear movement. (A) The equipment utilized to record mice whilst they took a drink from the water feeder. The data was set to use buffered video recordings, 10 s prior to a drink being taken and then for the next 30 s. (B) Overview of an occurrence of a mouse drinking from the water feeder.
[*Key*: a — regulator to control the pressure of 'air-puffs' delivered, b — USB 2.0 controlled valve programmed to release an air-puff for every 1/5 episodes from the water feeder that were randomized, c — the cooper floor implemented to ensure conductivity between the animal and the water feeder, d — water feeder and, e — USB link to computer software system (LabView).

30 cm high walls. At one end, a rectangular extension was added measuring 10 cm long, 4 cm wide, and 30 cm high. At the far end of this extension, a 5 mm hole was made so that a bottle water feeder with a metal spout could be mounted and allow the animal free access to water. A copper-conductive plate was mounted on the floor of the extension; this was connected by conductive wiring to a copper collar placed on the drinking spout. A second 3 mm diameter hole was made adjacent to the water feeder to permit the passage of a silicon tube. The purpose of this was to direct a puff of laboratory oxygen, regulated at 90 mmHg, at the face of the animal (Hadlock et al., 2010; Heaton et al., 2008). In the absence of a head restraint, the air-puff system caused an exaggerated startle response, rendering video footage unusable and therefore, was abandoned in this study. Anecdotally, mice were observed to retract their ears posteriorly whilst drinking from the water fountain, prompting this to be a behavioral process to investigate. A USB 2.0-powered high-definition monochrome digital camera with an in-built Progressive Scan CMOS CCD image sensor (ImagingSource DMK 72BUC02) fitted with a macro lens (Pentax™ TV lens 16 mm F1.4) was mounted to a fixed height of 45 cm above the floor of the extended section of the cage to record the animal as it drank from the water feeder. The camera was set to record 1024 × 768(Y800) at 45 frames per second.

The system was set up so that when the animal was drinking from the water feeder, an electrical circuit was established with the animal providing conduction between the copper floor pad and the copper collar around the water feeder. The purpose of this was to prompt the computer to save the video recording ten seconds prior to the connection being made and continue saving the recording for a total of thirty seconds. MATLAB code was written to permit the investigator to leave the animal unattended for several hours at a time and to ensure only 30 s of buffered video were captured around a drinking event, avoiding the need to manually screen through hours of empty footage. To improve the frequency mice went to drink from the water feeder, water was replaced with chocolate milk (Nesquik®, Nestle, Switzerland); this practice was adopted in all future video capture sessions. An overview of this system is shown in Fig. 4.

2.2. Surgical intervention

Female YFP-16 mice were chosen at six-weeks of age and were assigned to three groups. Each group contained ten animals with

the exception of the sham group, which contained five animals: sham group (Sh, n = 5), nerve excision group (Ex, n = 10), Nerve crush group (Cr, n = 10). All interventions were performed on the right PAN. The left ear served as the control for each animal.

Before and after surgery, all animals were allowed access to food and water ad libitum. All animals were anaesthetized with an intra-peritoneal injection of ketamine and xylazine (87 mg/kg of ketamine and 13 mg/kg of xylazine, dose = 0.1 ml/20 g). Once adequate anesthesia was confirmed, the area surrounding the right auricle was prepared for surgery by removing the fur around the surgical field with Nair™ (Church & Dwight Co., USA) after 3 min of application.

The surgical site was prepared using 70% ethanol solution. A 15 mm curvilinear incision was made extending from the posterior auricular margin towards the angle of the mandible. Surgical dissection proceeded as described in '1.4 Preliminary study: Function of the posterior auricular nerve' under a fluorescent operating microscope to expose and define the PAN. In all groups, the nerve was dissected freely and identification of the nerve was confirmed using a nerve stimulator (Vari-Stim® III, Medtronic, Xomed Inc.). The following steps were taken for each group as indicated:

Shamgroup$(Sh, n = 5)$:

After identifying the nerve, the surgical field was irrigated with 10 ml of normal saline solution (0.9% NaCl). No further manipulation of the PAN was performed.

Excisiongroup$(Ex, n = 10)$:

After identifying the PAN, a 3 mm section of the nerve was sharply excised and preserved in 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) solution. Confirmation of a nerve gap was verified using the fluorescent microscope to demonstrate a non-fluorescent gap in the nerve (Fig. 5). A 2 mm epineural incision was made in the facial nerve trunk and the proximal stump of the PAN was sutured in an end-to-side fashion to the facial nerve trunk using four 11-0 Ethilon® interrupted sutures (Ethicon, USA) at the 12, 3, 6 and 9 o'clock positions; this was to prevent the PAN from potentially neurotizing the interscutularis muscle as it regenerated from the proximal stump. The surgical field was irrigated with 10 ml of normal saline solution (0.9% NaCl).

Crushgroup$(Cr, n = 10)$:

After identifying the nerve, the PAN was crushed 3 mm from the origin of the facial nerve trunk. Size 5 jeweler's forceps were used to squeeze the nerve between the tips of the forceps for two periods of twenty seconds. Confirmation of an effective crush injury was verified using the fluorescent microscope to demonstrate a non-fluorescent gap in the nerve. The surgical field was irrigated with 10 ml of normal saline solution (0.9% NaCl).

In all animals, the surgical incision was closed using 5-0 Vicryl Rapide™ (Ethicon, USA) interrupted sutures. All animals received a subcutaneous injection of 0.5 ml of warmed 0.9% NaCl for rehydration, and a subcutaneous injection of buprenorphine (0.05 mg/kg) for post-operative pain control. Animals were recovered from anesthesia in a heated chamber (32–36 °C) with 95% oxygen, and observed continuously until they become sternally recumbent, in control of their airways and could eat and drink on their own. Experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) PHS Policy on Humane Care and Use of Laboratory Animals, the Animal Welfare Act (7 U.S.C. *et seq.*), and an animal use protocol approved by the Standing Committee on the Use of Animals in Research and Training of Harvard University (Protocol 24-08). At the end of the data collection period, all animals were euthanized in a CO_2 chamber.

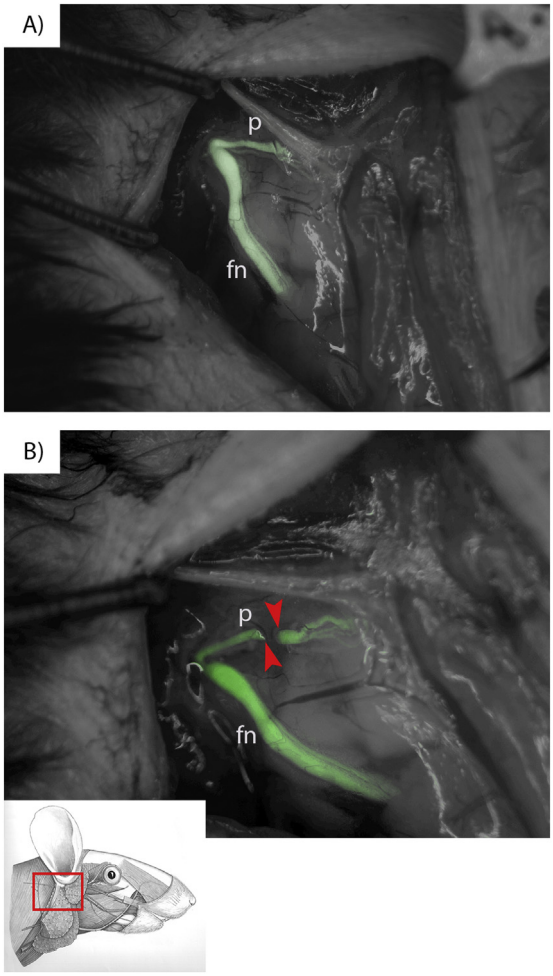

Fig. 5. Surgical procedures on the posterior auricular nerve. (A) Curvilinear incision around the base of the auricle exposes the posterior auricular nerve. In the sham group, the nerve was skeletonized with no added intervention. (B) The posterior auricular nerve was crushed until no fluorescence was visualized (between the red arrowheads). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
[*Key*: fn — facial nerve trunk, p — posterior auricular nerve].

2.3. Analysis of post-operative video-capture data

For all animals, a pre-operative baseline recording of their ear movement during drinking was captured. A data-captured session was complete once ten episodes of the animal drinking were recorded which was typically accomplished in a one-hour time period; this also served to allow the animals to acclimatize to the video-suite.

Subsequent video recording sessions were made for all groups post-operatively. On post-operative days (POD) 1, 10, 20 and 30, animals in all groups were recorded in the video suite drinking for a minimum of ten occasions. For each animal, a freeze frame was obtained from the buffered recording when the animal drank from the feeder; these were exported as. TIF files to Adobe Photoshop. To determine the change in ear movement, the right ear (interventional side) was compared to the left side (control side). Using the imaging software, a line (the 'inter-canthal line') was drawn linking the medial canthus of the right and left eyes. Two further lines (the 'posterior auricular lines') were drawn parallel to the inter-canthal line, one was placed at the most posterior point of the left auricle and one placed at the most posterior point of the right auricle. The distance was measured, in numbers of pixels, between the two posterior auricular lines and the inter-canthal line (Fig. 6). To calculate

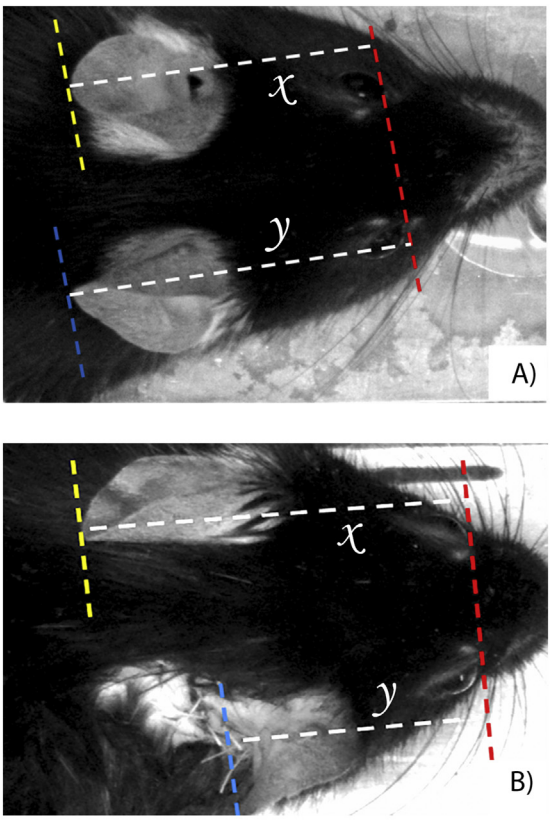

Fig. 6. Calculation of ear movement ratio. (A) Pre-operative measurement of both ears using the left ear as the control. The yellow line represents the normal position of the control ear during ear retraction, x is the distance (measured in pixels) from the inter-canthal line. The blue line represents the amount of retraction of the right ear from the inter-canthal line (y). (B) POD01 demonstrates the right ear in a fixed forward position following crush injury of the PAN nerve; the improvement in retraction was expressed as the ratio of recovery with respect to the left ear.

the ratio difference between the movements of the two ears the following equation was used in which x = distance left ear moved (control) and y = distance right ear moved (intervention):

$$\textit{ratio of recovery} = \left\{\frac{(x-(x-y))}{x}\right\}$$

The results were analyzed using GraphPad Prism5® (GraphPad Software Inc. USA). Student's paired *t*-test was used to compare the results between the groups and a 2-way analysis of variance (ANOVA) test was used to analyze each individual group.

3. Results

All 20 animals survived for the duration of the study and there were no complications from the surgical procedures. The animals tolerated the video capture sessions without any negative behavioral outcomes and all animals achieved recordings pre-operatively and at POD01, 10, 20 and 30 that captured ten occasions of drinking from the feeder.

Sham group (Sh, n = 5): animals exhibited a statistically significant reduction in right ear movement at POD01 in comparison to all other POD recordings ($p < 0.05$). From POD05 onwards, there was no difference between ear movements on either side, and there was no significant change in right ear movement during the study period ($p = 0.77$).

Excision group (Ex, n = 10): all animals had a significant reduction in right ear movement at all PODs in comparison to the left ear ($p < 0.05$). Further analysis failed to demonstrate any change in right ear movement during the study period ($p = 0.94$).

Crush group (Cr, n = 10): there was a significant reduction in right ear movement in comparison to the left ear at POD01, POD10, and POD20 ($p < 0.05$). At POD30, there was no significant difference between the ear movement on either side ($p = 0.35$). Two-way ANOVA analysis demonstrated a significant change in right ear movement during the data collection period ($p < 0.05$).

A summary of this data can be seen in Fig. 7.

4. Discussion

Most reports of outcomes following surgery use three distinct animal groups; a sham group, a nerve crush group and a transection or excision group; the understanding is that this best simulates injuries in the form of neuropraxia and neurotmesis (Dun and Parkinson, 2018; Fan et al., 2015; Kang and Lichtman, 2013; Kocaoglu et al., 2017; Wessel et al., 2017; Yu et al., 2014). Because the outcomes of sham, crushed nerve, and excised nerve groups are already well-established, this study evaluated the ability of PAN injury and interscutularis function to reflect such outcomes. The results of this study have demonstrated that assessment of ear movement can successfully differentiate between the three aforementioned experimental groups over the study period.

The sham group was selected to demonstrate any unforeseen consequences of surgery in the region of the PAN, such as damage to musculoskeletal structures that may affect ear movement. Indeed, at POD01 there was a significantly reduced level of movement between the right ear and the contra-lateral control side. Movement returned to normal, and no difference was demonstrated in the remaining study period. The fact that ear movement recovered to normal by POD10 suggests that no inadvertent damage took place to compromise ear movement. One proposed rationale for this difference is that at POD01, the animal is still recovering from the immediate local sequelae of surgery, such as pain, and that the reduced movement may be unrelated to the nerve. A second explanation is that a slight neuropraxia has developed as a consequence of intra-operative handling of the PAN during surgical dissection.

The excision group served as a negative control to ensure that the PAN was responsible for the assessed ear movement. To ensure there was no inadvertent neurotization of the muscle, a segmental excision was completed and an end-to-side neurorrhaphy was performed to redirect the PAN to grow along the facial nerve trunk. There was a significantly reduced level of ear movement at all PODs and a failure to demonstrate any level of recovery. The results from this group confirmed that the PAN was responsible for the ear movement being evaluated.

The crush group was included as the outcome of a crush injury is predictable. Previous rodent studies have reported that full recovery from crush injuries of the sciatic, tibial, phrenic, and facial nerves can be expected within the range of 21–28 days (Dun and Parkinson, 2018; Kang and Lichtman, 2013; Wessel et al., 2017; Wood et al., 2011), justifying the length of the data collection period for this pilot. This pilot study successfully demonstrated that the crush group recovered to normal function by POD30, with no statistical difference both between the movement of each of the animal's ears and the movement observed between the crush and sham groups. This result agrees with and confirms previous reports concerning motor recovery following crush injuries. A second important result was that a significant change in movement was observed during the study period. There was a graduated change in the recovery of ear movement at each POD that was statistically significant, suggesting that this methodology is sensitive enough to track changes in the recovery of movement.

As this study was primarily designed to evaluate whether the function of the posterior auricular nerve was measurable, a com-

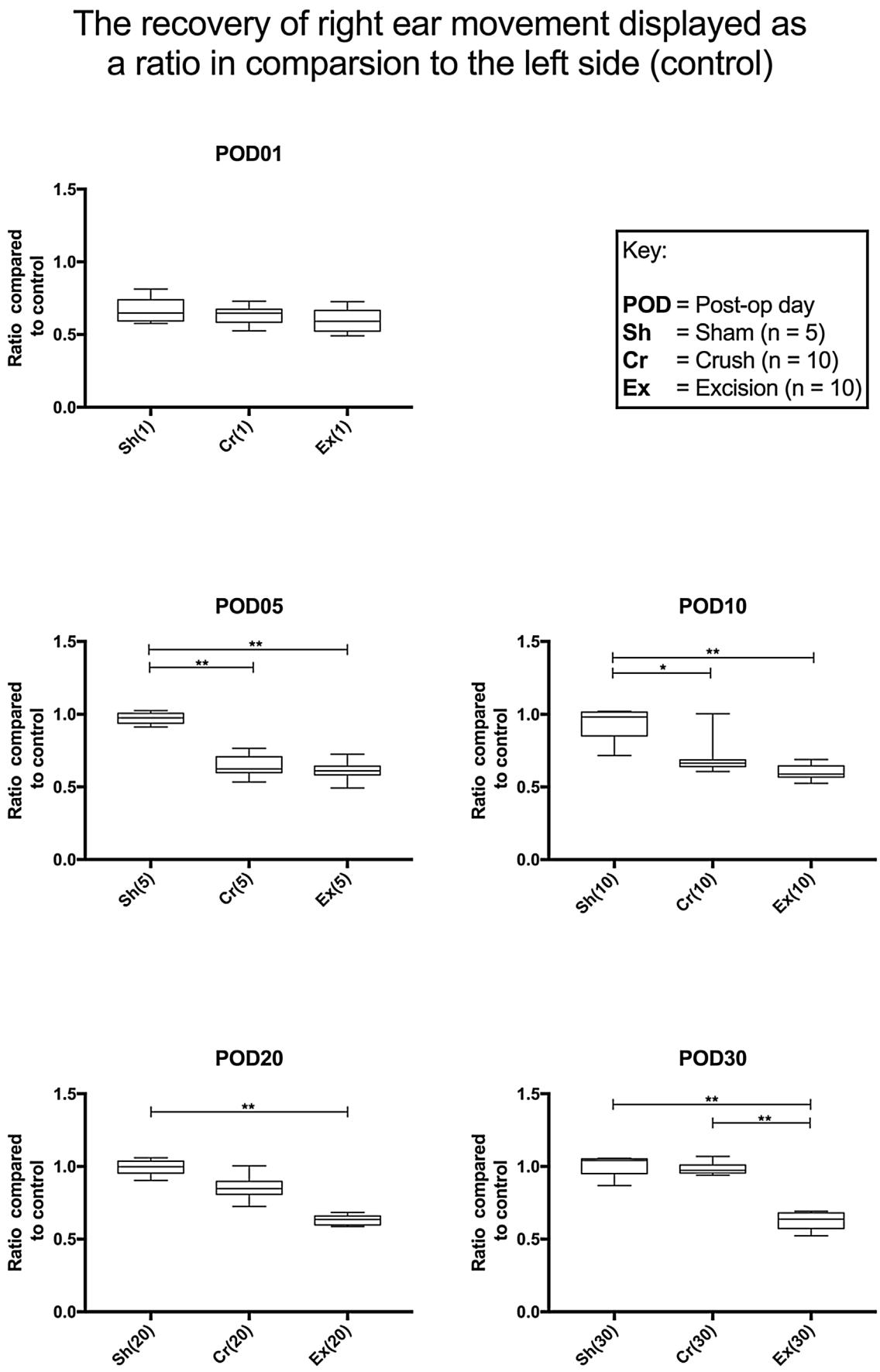

Fig. 7. Pilot study results of ear recovery following posterior auricular nerve manipulation.
During the study period, the crush group demonstrated a significant recovery in ear movement in comparison to the sham and excision groups. The results suggest that ear movement is a reliable assessment measure of functional outcome following facial nerve surgery.

plete transection of the facial nerve was not performed. For this study, an isolated transection of the posterior auricular nerve was required in order to evaluate and measure its isolated function. Work by Lu et al. and the investigators' previous work, discussed in 1.3, and 1.4, demonstrates that the posterior auricular nerve receives no collateral innervation along its path from the branch point to the auricle (Lu et al., 2009). Therefore, the posterior auricular nerve is supplied only by the facial nerve, and complete transection of the facial nerve would result in failure of posterior auricular nerve function. That is, if a complete transection of the facial nerve was undertaken, such work would require the completion of an additional experiment with additional animal sacrifice and would be unlikely to alter the impact of our results. However, functional impact over time with reinnervation is unknown; a complete lesion proximal to the PAN may lead to synkinetic reinnervation of the ear muscles. A pure PAN lesion, such as was created in this experiment, may not be ideal for synkinetic reinnervation studies.

Based upon the analysis completed in this study, the PAN appears to be an excellent nerve to use for facial reanimation surgery evaluation: there is no cross-innervation and outcomes are measurable by monitoring ear movement. Additionally, a recent study has demonstrated a correlation between ear posture and emotional reactivity in mice (Lecorps and Feron, 2015), making the analysis of ear movement even more appropriate; it is controlled by the facial nerve and is an expression of emotion much like the action of smiling in humans. Furthermore, these measures are non-restrictive and less emotionally taxing for the animals than the evaluation of whisking, for example, which may require head fixation.

5. Conclusion

The aim of this study was to determine whether ear movement could be analyzed to produce a viable outcome measure in facial nerve surgery. The results show that significant differences were demonstrated between the experimental groups and that significant changes within the crush group were identifiable, making this an acceptable model to develop as an accurate outcome measure following rodent facial nerve surgery.

Acknowledgement

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